THE ADSORPTION OF BACTERIA BY MARINE BOTTOM[1]

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In various recent studies (1, 4, 5) on the adsorption of bacteria in soil, the impression is left that the bacteria exert, in that state, only little effect upon the cycle of life in the soil. In his book on Agricultural Microbiology, Chudiakov (2) states emphatically that the problem of the condition of the bacteria in the soil hampered considerably a better understanding of soil microbiological processes. The existence of a surface relationship between the soil and microörganisms is true not only of bacteria but also of Protozoa, as shown by Cutler (3).

Different species of bacteria were found to behave differently in regard to their adsorption by the soil particles; their mobility and ability to form zoöglea seemed to be of special significance in this connection. The chemical activities of the bacteria, as measured by the evolution of CO_2, were considerably modified by the adsorption process (4). The finer soil constituents, namely the clay and silt fractions, were found to have a much greater adsorptive effect than the sand. Different soil types were found to adsorb bacteria to a different degree (5).

Rubentschik (8) made a detailed study of the phenomenon of bacterial adsorption in salt basins; the bacteria isolated from the mud were found to show a higher degree of adsorption than the bacteria found in the water. The conclusion was reached that the bacterial benthos consist of easily adsorbable species whereas the bacterial plankton contains organisms possessing a low degree of adsorption by the bottom sediments. A bacterial exchange took place in the mud, the adsorption of some species being accompanied by the desorption of others from the mud. The relative concentration of the bacteria, their nature and the type of bottom material exerted an influence upon the adsorption process. The activities of different species of bacteria were variously modified in the adsorbed state: the metabolism of some was lowered and that of others was increased.

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According to Peele (6), the adsorption of bacteria is probably due to the attraction of unlike electric charges. The nature of the base in the soil complex influences considerably the process of adsorption, the monovalent cations showing the least adsorption.

In most of these studies, pure cultures of bacteria were used and the results obtained interpreted in terms of processes carried out by a complex population of microörganisms inhabiting the soil or the sea bottom. Usually a very short period of contact was allowed between the soil and the bacterial culture grown upon an artificial medium; no attempt was made to determine what happened after the culture had adjusted itself to the environment.

These studies have an important bearing upon the influence of the sea bottom upon the bacterial activities in the water. A number of questions may arise in this connection; 1. Is the specific occurrence of certain bacteria in the bottom due to their adsorption by the bottom material? 2. Is the relatively low number of bacteria in the sea water, as compared with that in the bottom, due to their removal from the water by the adsorption process? 3. Is this relationship responsible for the difference in the rate of bacterial processes taking place in shallow seas over a sand bottom such as Georges Bank, compared with the corresponding processes over a mud bottom, such as the Gulf of Maine (7, 11)?

These and other questions deserve fundamental treatment. No attempt will be made to give a definite answer in the following experiments, which must be considered as preliminary in nature.

Experimental

In order to eliminate the interfering effect of the bacterial population normally found in fresh bottom material, the first experiments were carried out with marine mud and sand which had been kept in a dry state for 5 years and in which the bacteria had been reduced to very low numbers.

In the first experiment, the adsorption of bacteria from a mixed population and from a pure culture of a marine bacterium by two different types of dry marine mud and sand was studied. A mixed culture was obtained by allowing fresh sea water to remain, in a glass container, in the laboratory, for a period of 48 hours. This resulted in an increase in the numbers of bacteria from a few hundred to 303,000 per 1 cc., as determined by the plate method. As a pure culture, a marine agar liquefying bacterium (No. 11) was used. This organism was selected because of the ease of recognizing the colonies produced on the plate. It had been isolated from sea water and kept in culture for a period of 2 years. The organism was grown in a medium poor in nutrients (1 gram peptone, 1 gram glucose and 0.5 gram K_2HPO_4 in one liter of sea water), for 24 or 48 hours. The culture was then diluted ten times with sea water sterilized by heating 30 minutes at 80° C. The two muds, No. 1329 and No. 1331, contained 2.46 and 1.58 per cent organic carbon and 0.28 and 0.16 per cent nitrogen, respectively (9). The sand contained 0.58 per cent carbon and 0.05 per cent nitrogen. Ten-gram portions of mud or sand were placed in 250-cc. flasks containing either 100 cc. cultured sea water or 100 cc. of the diluted 24-hour culture of the bacterium. The flasks were shaken by hand for 10 minutes, allowed to stand 10 minutes, and 1-cc. portions of the supernatant liquid plated out using a sea water agar medium (No. 1). The

TABLE I

Adsorption of bacteria from mixed and pure cultures by dry marine mud and sand

Nature of culture	Bacteria in 1 cc. water, thousands			
	Start	*10 minutes*	*2 hours*	*21 hours*
Cultured water				
Control	303	—	—	—
Plus mud 1329	—	28	13	3,700
Plus mud 1331	—	25	75	530
Plus sand	—	180	115	32,000
Bacterium No. 11				
Control	1,600	—	—	—
Plus mud 1329	—	9,620	6	5
Plus mud 1331	—	13,000	21	365
Plus sand	—	22,000	40,000	40,000

flasks were kept in the laboratory, with occasional shaking, and water plated out again after 2 and 21 hours.

The results presented in Table I show that the pure culture of the bacterium was readily adsorbed by the marine mud. The sand, however, had no adsorptive effect; there was, in fact, a marked increase in bacterial numbers due to the separation of the bacterial masses or clumps into individual cells as a result of shaking with the sand. In the case of the crude cultures (i.e. the bacterial population in the cultured sea water), the immediate adsorption of the bacteria was followed by a rapid increase in numbers. The latter was due to the rapid multiplication of certain bacterial forms at the expense of the organic matter in the mud and sand.

This experiment was repeated and an attempt made to measure not only the multiplication of the bacteria, but also their activities, as influenced by the presence of bottom material. This could best be done by determining the oxygen absorption, as a result of bacterial multiplication (10). Two-gram portions of the two muds and 5-gram portions of the sand were placed in oxygen bottles of about 220 cc. capacity. The bottles were filled with 3-day-old cultured sea water. They were closed and incubated at room temperature, under water and in the dark. At different intervals, some of the bottles were removed and analyzed for bacteria, by plating out 1 cc. of the supernatant water, and for oxygen by the Winkler method.

The results reported in Table II show that there was comparatively little adsorption of bacteria from the mixed bacterial population in cultured sea water, by either marine mud or sand. On the contrary, there was a marked rise in bacterial numbers, reaching a peak in 17 to

TABLE II

Influence of marine bottom material on the growth and metabolic activity of a bacterial sea water population

Nature of culture	Bacteria in 1 cc. water, thousands					
	Start	*3 hours*	*17 hours*	*42 hours*	*4 days*	*8 days*
Sea water control	0.5	0.9	1.2	1.0	7.3	3.1
Plus mud 1329	1.8	1.9	1,175.0	850.0	133.0	12.2
Plus mud 1331	1.9	1.7	1,240.0	1,435.0	205.0	3.3
Plus sand	2.2	3.9	1,255.0	615.0	65.0	3.3
	Oxygen consumed, cc. per liter					
	Start	*3 hours*	*17 hours*	*42 hours*	*4 days*	*8 days*
Sea water control	0.0	0.03	0.07	0.23	0.38	0.64
Plus mud 1329	0.0	0.25	0.73	1.42	2.53	3.48
Plus mud 1331	0.0	0.13	0.62	1.29	2.47	3.31
Plus sand	0.0	0.19	0.88	1.57	2.18	3.24

42 hours, followed by a rapid decline. Active oxygen absorption took place in the bottles accompanying bacterial multiplication; this was due to the oxidation of the organic matter in the bottom material by the bacteria.

A study was now made of the adsorption of bacteria from mixed and pure cultures by the use of relatively large amounts of bottom material. In this experiment, the mud and sand were placed in large test tubes and sterilized by heat at 120° C. for 1 hour. Two volumes of cultured water, 2 days old, or two volumes of a diluted 2-day-old culture of No. 11 were added to the sterile tubes. These were now shaken for 1 minute and allowed to settle for 10 minutes. The supernatant liquid was plated out immediately. The tubes were then allowed to remain in the laboratory for 24 hours; some were shaken occasionally and some were left undisturbed. The water was plated out after 24 hours. The results (Table III) show that there was again a rapid adsorption of the bacteria from the pure culture by the mud but not by the sand. In the mixed culture there was at first a certain amount of adsorption of the bacteria; this was followed by a very rapid increase in numbers. The large amount of mud used offered a good source of energy, especially after heating, for many of the bacteria in the water.

A comparative study was now made of the adsorption of bacteria by fresh marine mud obtained from Buzzards Bay. Varying amounts

TABLE III

Influence of shaking upon the adsorption of mixed and pure cultures of bacteria by sterile marine bottom material

Nature of culture	Bacteria in 1 cc. of surface water, thousands		
	Shaken		Unshaken
	Start	*24 hours*	*24 hours*
Cultured water control	4.6	153.0	170.0
Plus mud 1329	0.3	3,600.0	39,000.0
Plus mud 1331	0.1	12,000.0	22,000.0
Plus sand	100.0	10,000.0	7,000.0
Bacterium No. 11 * control	33,000.0	214,000.0	130,000.0
Plus mud 1329	3,300.0	4,400.0	31,000.0
Plus mud 1331	30.0	1.0	1.0
Plus sand	39,000.0	320,000.0	220,000.0
Bacterium No. 11 † control	13,000.0	21,000.0	21,000.0
Plus mud 1329	0.7	1.0	1.0
Plus mud 1331	30.0	1.0	1.0
Plus sand	18,000.0	18,000.0	25,000.0

* Dilution of culture with sterile sea water in ratio of 1 : 10.
† Dilution of culture in ratio of 1 : 30.

of the fresh mud, containing 75 per cent moisture, were placed in 250-cc. Erlenmeyer flasks. Some flasks received 100 cc. portions of 2-day-old sea water, while other flasks received 100 cc. of a diluted 2-day-old culture of No. 11. All flasks were shaken for 1 minute, allowed to settle for 10 minutes and the supernatant liquid plated. This was repeated after 1, 2, 4 and 10 days. Some of the flasks were kept undisturbed, while others were shaken at various intervals. The plates were incubated at room temperature for 48 hours and counted.

The results (Table IV) show that fresh marine mud exerted at first a reducing effect upon the numbers of bacteria in the mixed population.

After 24 hours, however, there were more bacteria in the water above the mud than in the control water sample. It is interesting to note that the larger the amount of mud used, the higher was the number of bacteria, especially in the shaken mud, in spite of the fact that the immediate reduction in numbers was greater with an increase in the amount of mud. This was due to the fact that the bacteria found in the mud a source of nutrients and began to multiply rapidly. This confirms the results obtained previously by the use of dry mud and sand.

In the case of the pure culture, there was active adsorption of the

TABLE IV

Adsorption of bacteria by fresh marine mud

Nature of culture	Fresh mud *	Shaking	Numbers of bacteria in 1 cc. water, thousands				
	grams per 100 cc.		*Start*	*24 hours*	*48 hours*	*4 days*	*10 days*
Cultured water .	0	0	53	10	9	9	<1
Cultured water .	2	0	44	17	4	3	<1
Cultured water .	10	0	42	10	32	23	6
Cultured water .	50	0	24	32	50	13	2
Cultured water .	0	+	45	16	11	21	<1
Cultured water .	2	+	30	13	27	29	17
Cultured water .	10	+	33	16	23	36	62
Cultured water .	50	+	23	13	21	79	80
No. 11.........	0	0	12,700	140,000	240,000	236,000	310,000
No. 11.........	2	0	11,900	23,000	3,000	2,500	58
No. 11.........	10	0	13,900	1,000	2,000	3,600	182
No. 11.........	50	0	17,900	5,900	6,000	1,800	41
No. 11.........	0	+	14,700	160,000	332,000	310,000	360,000
No. 11.........	2	+	16,600	67,000	23,000	6,700	160
No. 11.........	10	+	15,400	13,000	6,000	1,100	92
No. 11.........	50	+	15,000	4,400	1,000	68	104

* Moisture content of mud 75 per cent.

bacteria by the fresh marine mud, but here also the bacteria began to multiply after 24–48 hours. These were not, however, the typical colonies of the agar liquefying organism added to the mud, but various colonies of bacteria commonly found in the marine bottom.

On the basis of these results, one can hardly conclude that the adsorptive action of the mud bottom upon bacteria in the water is detrimental in any manner to their activities. Even though mud adsorbs, removes, or inactivates a large number of bacterial cells such as may be obtained by growing a pure culture of a bacterium upon an artificial medium, there is still no proof that mud as such is in any manner injurious to bacterial processes in general, either those taking place in the water or in the mud itself. The evidence presented here seems to point merely to the fact that bottom mud exerts a certain controlling effect upon the nature of the bacterial population in the sea water. This is, of course, true of the bacteria found in the benthos or in the water immediately above it, but may not hold at all for the plankton bacteria found at great distances from the bottom.

The results of these experiments further emphasize the fact that the phenomena of adsorption obtained by the use of pure cultures of bacteria grown upon artificial media are difficult to interpret in terms of natural marine processes, since the conditions controlling the relations of the mud bottom to the natural mixed population of bacteria in sea water are much more complex.

Summary

1. Marine mud exerted an adsorptive effect upon the bacteria in sea water. This effect was particularly evident when certain pure cultures of bacteria were added to the mud to give a heavy inoculation. Sand bottom had very little adsorptive action upon either mixed or pure cultures of bacteria.

2. In the case of the mixed bacterial population in the water, the adsorption of the bacteria by the mud was soon followed by a rapid increase of bacterial numbers; this took place at the expense of the organic matter in the bottom material.

3. These laboratory experiments showed that certain types of marine bottom material exerted a controlling effect on the numbers of bacteria, which were in intimate contact with them. There was no indication, however, of a permanent paralysis of bacterial growth or metabolism.

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